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A new genus and two new species of freshwater mussels (Bivalvia, Unionidae) from Sichuan, China: Overlooked cryptic endemism in the upper Yangtze River Basin

Hui Chen[1], Hong-Quan Xiang[2], Yu-Ting Dai[1], Zhong-Guang Chen[1], Yue-Ming He[3], Ge Guo[4], Xiao-Ping Wu[1], Xiao-Chen Huang[1]

1 *School of Life Sciences, Nanchang University, Nanchang, 330031, China*
2 *Department of Animal Science, Yuxi Agriculture Vocation-Technical College, Yuxi, 653106, China*
3 *College of Engineering and Technology, Tianjin Agricultural University, Tianjin, 300384, China*
4 *Beijing Chaoyang RCF Dongba School, Beijing, 100018, China*

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Corresponding authors: Xiao-Ping Wu (xpwu@ncu.edu.cn); Xiao-Chen Huang (xchuang@ncu.edu.cn)

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Abstract

Freshwater mussels are ecologically vital but understudied in China's Sichuan Basin, a biodiversity hotspot with significant gaps in documented diversity. Here, we conducted an investigation into the Unionidae species in Dazhou, Sichuan Province, China, and discovered two species of Unionidae that had never been recorded before. This paper confirmed through mitochondrial phylogenomic analyses that they represent new species belonging to the genera *Pseudocuneopsis* Huang, Dai & Wu, 2022: *Pseudocuneopsis perflora* H. Chen, H.-Q. Xiang, Y.-M. He, X.-C. Huang & X.-P. Wu, **sp. nov.** and a new genus and species: *Tongchuania mingiana* H. Chen, H.-Q. Xiang, Y.-M. He, X.-C. Huang & X.-P. Wu, **gen. et sp. nov.** Additionally, the shell morphology and soft-body anatomical characteristics of the two new species was described. These discoveries not only expand the known diversity of Unionidae in the upper Yangtze River Basin but also highlight the Sichuan Basin as a potential center of cryptic endemism. Our findings underscore the importance of combining molecular and morphological methods for uncovering hidden diversity in understudied regions and provide critical data for the conservation of China's imperiled freshwater mussel fauna.

Key Words

Freshwater mussels, Mollusca, Sichuan Basin, taxonomy, Unionini

Introduction

Freshwater mussels (Bivalvia, Unionida) play a critical role in maintaining the ecological integrity and stability of freshwater ecosystems (Huang et al. 2019; Liu et al. 2022; Zieritz et al. 2022; Dai et al. 2025). East Asia is recognized as a global biodiversity hotspot for unionid biodiversity (Graf and Cummings 2021), with China harboring particularly high species richness and endemism (Zieritz et al. 2018; Liu et al. 2022). Despite considerable efforts to document Chinese freshwater mussel diversity (Liu et al. 1979; Guo 2022), the continuous discovery of new genera and species highlights the incompleteness of current taxonomy, largely due to recent advances in integrative methods combining molecular phylogenetics and morphology (Chen et al. 2023; Dai et al. 2023; Dai et al. 2024a, 2024b; Dai et al. 2025; Huang et al. 2025). Many of these discoveries have emerged from previously underexplored regions, such as recent surveys in the middle and lower reaches of the Yangtze River and the Pearl River Basin (Xiong et al. 2011, 2012; Xiao et al. 2012; Dai et al. 2024a, 2024b). However, the upper reaches of the Yangtze River, with particular emphasis on the Sichuan Basin, remain largely understudied, resulting in

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significant gaps in our knowledge of both regional diversity and the broader picture of global unionid diversity.

The Sichuan Basin, a vast intramontane basin with a highly complex geological composition situated in the upper Yangtze River Basin, harbors a dense network of rivers that likely supports a rich but poorly documented unionid fauna (Zeng et al. 1981, 1985). Compared to adjacent regions, despite the known global diversity of Unionidae (Graf and Cummings 2007), the documented diversity of freshwater mussels in the basin appears strikingly low, only four endemic species formally recorded to date: *Acudonta baitiaoensis* Wu, Liu & Zhang, 2025; *Pseudocuneopsis sichuanensis* Huang, Dai & Wu, 2022; *Acuticosta sichuanica* Zeng & Liu, 1989; and *Parvasolenaia triangularis* (Heude, 1885) (Heude 1885; Zeng and Liu 1989; Wu et al. 2022, 2025). This apparent paucity may reflect either insufficient sampling or the presence of cryptic lineages that remain unrecognized. Consequently, the Sichuan Basin is increasingly viewed not merely as a potential center of hidden endemism, but rather as a key region for the diversification and evolution of freshwater mussel species, awaiting further exploration.

In this study, we described two new taxa from Dazhou City, Sichuan Province, China: *Pseudocuneopsis perflora* sp. nov. and *Tongchuania mingiana* gen. et sp. nov., both of which occur sympatrically in the same river system. By integrating mitochondrial genomic data with morphological evidence, we established their taxonomic status and revealed previously overlooked diversity among freshwater mussels in the upper reaches of the Yangtze River. These findings underscored the urgent need for expanded field surveys and integrative taxonomic research in understudied regions to fully elucidate the evolutionary and ecological complexity of freshwater mussel fauna.

Materials and methods

Specimen sampling and morphological observations

Freshwater mussels were collected in Bahe River (31.0978°N, 107.1856°E), Dazhou City, Sichuan Province, China, in April 2025 (Fig. 1). The specimens were preserved in 95% ethanol, and deposited at the Museum of Biology, Nanchang University (NCUMB), China. A Vernier caliper was used to measure shell length (L), shell width (W) and shell height (H).

DNA extraction, PCR, sequencing and mitogenome assembly

Total genomic DNA was extracted on foot tissues using TIANamp Marine Animals DNA Kit (Tiangen Biotech) following the manufacturer's protocol. F-type mitochondrial COI sequences were amplified using primers LCO22me2 (GGTCAACAAAYCATAARGATATTGG) and HCO700dy2 (TCAGGGTGACCAAAAAAYCA) (Walker et al. 2007). Polymerase chain reaction (PCR) amplifications of COI were performed in a final 25 μL volume mixture containing 1 μL of template DNA, 1 μL of each pair of primers, 12.5 μL of Green Taq Mix (Vazyme, China), and 9.5 μL ddH2O. Thermal cycling began with one cycle at 98 °C for 10 s, followed by 35 cycles of denaturation at 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, with a final extension step at 72 °C for 7 min. The PCR products were purified and sequenced using an ABI 3730XL analyzer. The newly obtained sequences were deposited in GenBank (Suppl. material 1: tables S1, S2).

F-type mitogenomes were obtained in this study, Qualified genomic DNA samples were dispatched to Novogene (Beijing, China) for library construction and sequencing. After discarding low quality reads, clean reads were obtained and assembled de novo by CLC Genomic Workbench v. 12.0. Contigs identified as mitogenome sequences were checked manually for overlap at the beginning and end, resulting in a circular mitogenome. The newly produced mitogenome was checked manually and annotated with Geneious v.11.0 (Kearse et al. 2012). The initial annotation of the mitogenome was carried out using the MITOS web server (Bernt et al. 2013). ARWEN (Laslett and Canback 2008) was also used to identify the locations of all tRNA genes. The annotations for two rRNA genes were further refined based on the positions of neighbouring genes. Open Reading Frame Finder (http://www.ncbi.nlm.nih.gov/orffinder/) and BLAST searches (http://blast.ncbi.nlm.nih.gov/) were used to identify 13 protein-coding genes (PCGs).

Alignments and partitioning strategies

Mitogenomic dataset were constructed in this study (including 12 PCGs excluding ATP8, and two rRNA genes; Suppl. material 1: table S3). Sequences were aligned using MAFFT based on the Q-INS-i algorithm (ver. 7, see https://mafft.cbrc.jp/alignment/server/; Katoh et al. 2019). Pairwise distances between species were calculated using MEGA X (Kumar et al. 2018). PartitionFinder 2 (ver. 2.3.4, see http://www.robertlanfear.com/partitionfinder/; Lanfear et al. 2017) were used to select the best-fit partitioning schemes and models under greedy search with unlinked branch lengths (Suppl. material 1: table S4). Predefined data blocks for partitioning scheme searches were designated by gene region (rRNA gene) or codon position (PCG). Branch lengths were permitted to be unlinked, with the AICc method utilized to determine model selection and partitioning schemes.

Phylogenetic analyses

Bayesian inference (BI) and maximum likelihood (ML) analysis were performed using MrBayes (ver. 3.2.6, see http://nbisweden.github.io/MrBayes/; Ronquist et al. 2012) and IQ-TREE (ver. 2.2 Minh et al. 2020),

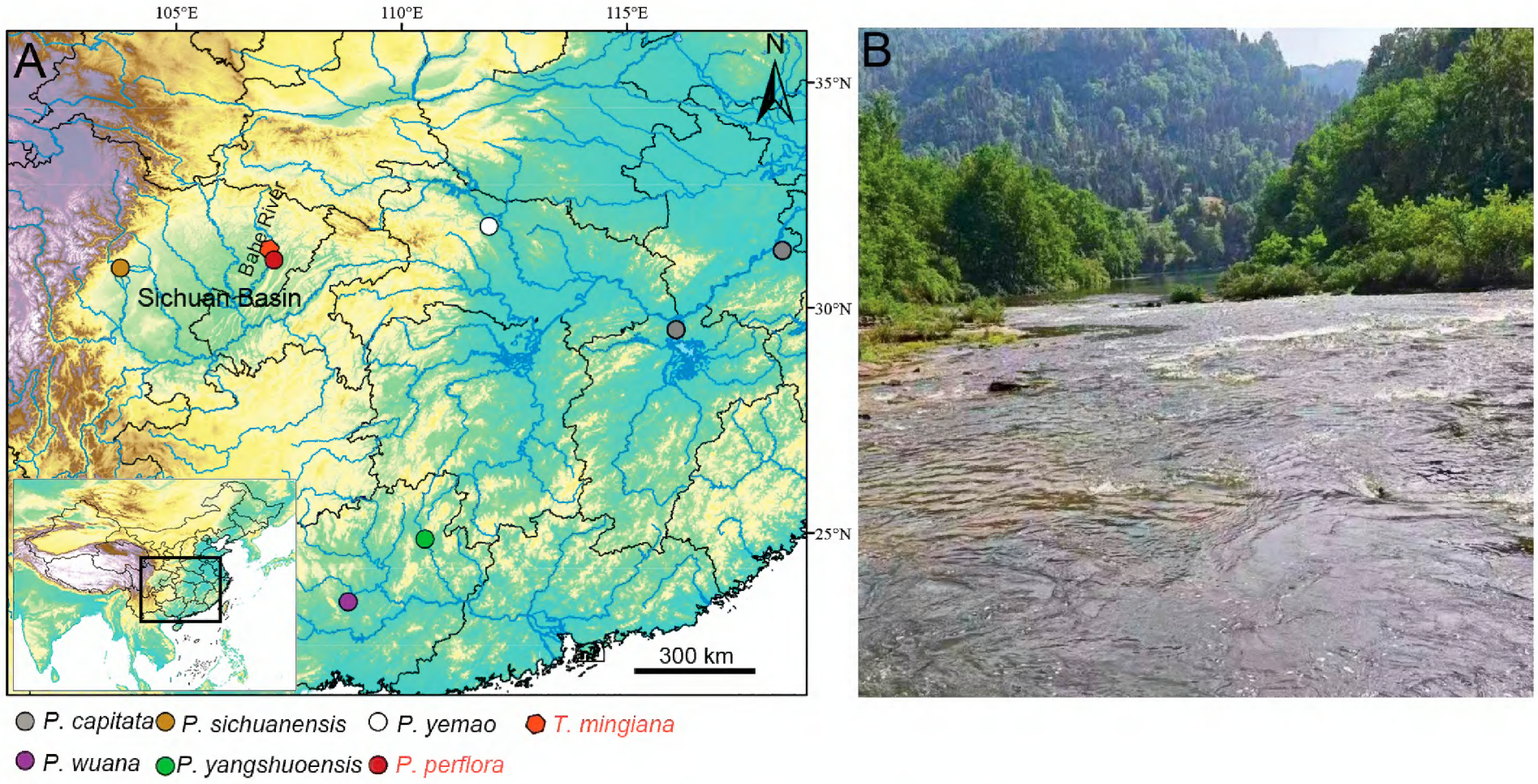

Figure 1. Distribution map of *Tongchuania mingiana* gen. et sp. nov. and *Pseudocuneopsis*.

respectively, with reference to the selected model of sequence evolution. For Bayesian analysis, two runs were performed simultaneously with four Markov chains starting from a random tree. Bayesian posterior probabilities (BPPs) of nodes were determined using Metropolis-coupled Markov chains (one cold chain) for 2,000,000 generations, with sampling every 1000 generations. The first 25% of sampled trees were discarded as burn-in when the standard deviation of split frequencies of the two runs was less than 0.01; the remaining trees were then used to create a 50% majority-rule consensus tree and to estimate BPPs. Node support for maximum likelihood analysis was determined using 1000 rapid bootstrap (BS) replicates.

Species delimitation analyses and genetic distance calculation

MEGA X (Kumar et al. 2018) was used to construct a neighbor-joining (NJ) tree and calculate genetic distances using uncorrected *p*-distance. We used three distinct approaches to determine the number of molecular operational taxonomic units (MOTUs). These approaches were the Poisson Tree Process model (bPTP) (Zhang et al. 2013), Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012) and haplotype network reconstructions in TCS (ver. 1.21, see http://en.bio-soft. net/tree/TCS.html; Clement et al. 2000). The bPTP web server (see http://species.h-its.org/) was used to perform the bPTP analysis on the NJ tree of 27 sequences. The analysis was conducted over 500,000 generations, with a thinning of every 500 generations and a 10% burn-in. The ABGD was run online (http://wwwabi.snv.jussieu.fr/public/abgd/) with the default settings and uncorrected *p*-distance matrix (Puillandre et al. 2012). Haplotype networks were reconstructed with 95% statistical parsimony in TCS. The number of haplotypes was calculated with DnaSP (ver. 6, see http://www.ub.edu/dnasp/; Rozas et al. 2017). The TCS network was created using PopART (ver. 1.7, see https://popart.otago.ac.nz/index.shtml; Leigh et al. 2015).

Results

Species delimitation analyses

The TCS network reflected the outcomes of the *Pseudocuneopsis* species delimitation analyses, revealing six distinct groups as shown in Fig. 2A. Species delimitation analysis using three methods identified six putative species within *Pseudocuneopsis* and the species found in Dazhou, Sichuan, China represents a distinct taxon (Fig. 2B). Additionally, pairwise uncorrected COI *p*-distance analysis demonstrated genetic distances between this species with other *Pseudocuneopsis* species ranging from 8.37% (between this species and *P. wuana*) to 10.12% (between this species and *P. capitata*) (Table 1). These distances, as well as the distinct morphological characteristics, provide evidence for its classification as a new species *Pseudocuneopsis perflora* sp. nov.

The COI *p*-distance between another species collected in the same location with other genera in the tribe Unionini, ranged from 10.13% (between this species and *Tchangsinaia piscicula* (Heude, 1874) to 14.02% (between this species and *Unio crassus* Philipsson, 1788). Among its closest relatives, the distances were 10.13% (*T. piscicula*), 10.66% (*Cuneopsis heudei*), 11.07% (*Schistodesmus spinosus*) and 11.67% (*Nodularia douglasiae*) (Table 2). These substantial genetic divergences support the establishment of the new genus. Furthermore, this species shows unique morphological characteristics distinguishable from other genera. Therefore, it is described herein as *Tongchuania mingiana* gen. et sp. nov.

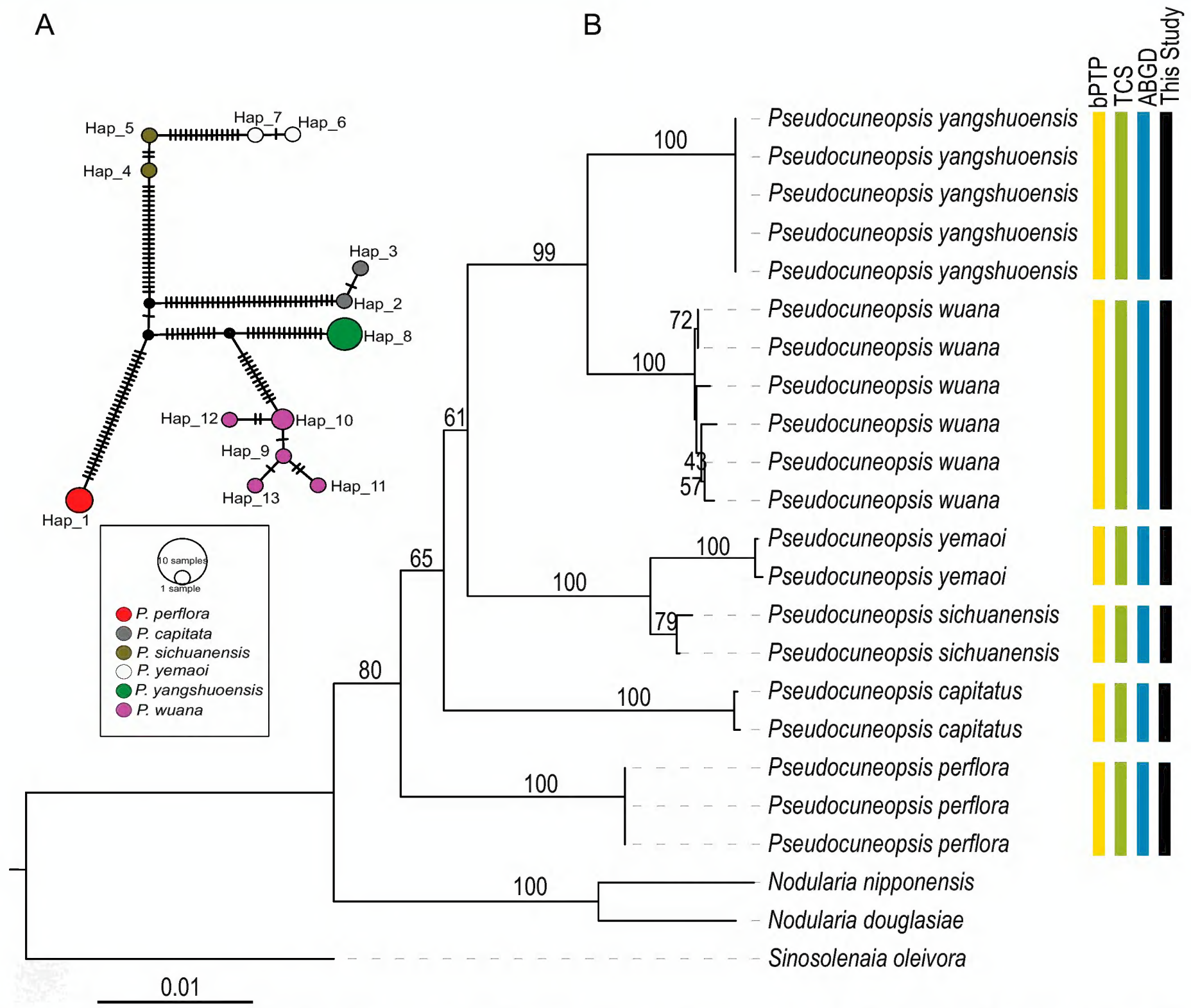

Figure 2. Analysis based on the COI dataset. **A.** The haplotype network was constructed from the CO1 haplotypes of the six *Pseudocuneopsis* species. Haplotype frequencies are related to the size of the circle; **B.** Neighbor-joining tree of 27 COI sequences based on uncorrected p- distance. Results from three delimitation analyses are shown, with bars colored to indicate groupings and to compare congruence between methods.

Table 1. The average p-distances (%) among species of *Pseudocuneopsis*.

	***P. perflora* sp. nov.(3)**	***P. wuana* (6)**	***P. yemaoi* (2)**	***P. yangshuoensis* (4)**	***P. sichuanensis* (2)**
P. perflora sp. nov. (3)					
P. wuana (6)	8.37				
P. yemaoi (2)	9.32	8.92			
P. yangshuoensis (4)	9.80	4.40	9.25		
P. sichuanensis (2)	8.68	7.43	2.36	7.78	
P. capitatus (2)	10.12	9.40	9.74	9.41	8.83

Note: The numbers in parentheses indicate sequenced individuals.

Table 2. Genetic differentiation by means of p-distances (%) between genera of tribe Unionini.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1. *Tongchuania mingiana* gen. et sp. nov.								
2. *Pseudocuneopsis perflora*	11.45							
3. *Nodularia douglasiae*	11.67	11.4						
4. *Unio crassus*	14.02	12.1	14.7					
5. *Schistodesmus spinosus*	11.07	10.38	11.21	12.9				
6. *Cuneopsis heudei*	10.66	12	12.13	15.08	11.32			
7. *Tchangsinaia piscicula*	10.13	10.07	11.96	13.22	12.23	11.1		
8. *Middendorffinaia mongolica*	10.65	9.73	10.76	12.18	10.22	10.86	11.95	
9. *Diaurora aurorea*	10.89	11.84	12.37	13.73	12.46	11.45	10.59	11.6

Mitochondrial phylogeny

The complete mitochondrial genomes for *Tongchuania mingiana* gen. et sp. nov. and *P. perflora* sp. nov. are 15,843 and 15,798 bp, respectively, all of which contained the typical 37 genes (13 PCGs, 2 rRNAs and 22 tRNAs) (Fig. 3A, B). The mitochondrial gene order was the same as that of the subfamily Unioninae (Wu et al. 2022). The maximum likelihood and Bayesian analyses produced largely consistent phylogenetic trees (Fig. 3C). Molecular phylogenetic analyses unequivocally placed *Tongchuania* gen. nov. within Unionini and revealed its close relationship with *Schistodesmus* as a sister group (posterior probabilities (PP) = 1.0, bootstrap (BS) = 100). This clade was sister to the clade including *Cuneopsis* and *Tchangsinaia* (PP = 1.0, BS = 100), and the entire group showed a sister relationship to *Nodularia* (PP = 1.0, BS = 76). The topology can be summarized as: ((*Tongchuania* + *Schistodesmus*) + (*Cuneopsis* + *Tchangsinaia*)) + *Nodularia*. This robust phylogenetic framework strongly supports the establishment of *Tongchuania* as a distinct genus while revealing its close evolutionary affinity with *Schistodesmus* among Unionini. Phylogenetic analysis also revealed a well-resolved clustering pattern within the genus *Pseudocuneopsis*, where *P. sichuanensis* and *P. yemaoi* formed a sister-group relationship (PP = 1.0, BS = 76), while *P. wuana* and *P. yangshuoensis* clustered together as a distinct clade (PP = 1.0, BS = 76). These four species collectively grouped with *P. perflora* sp. nov. as their

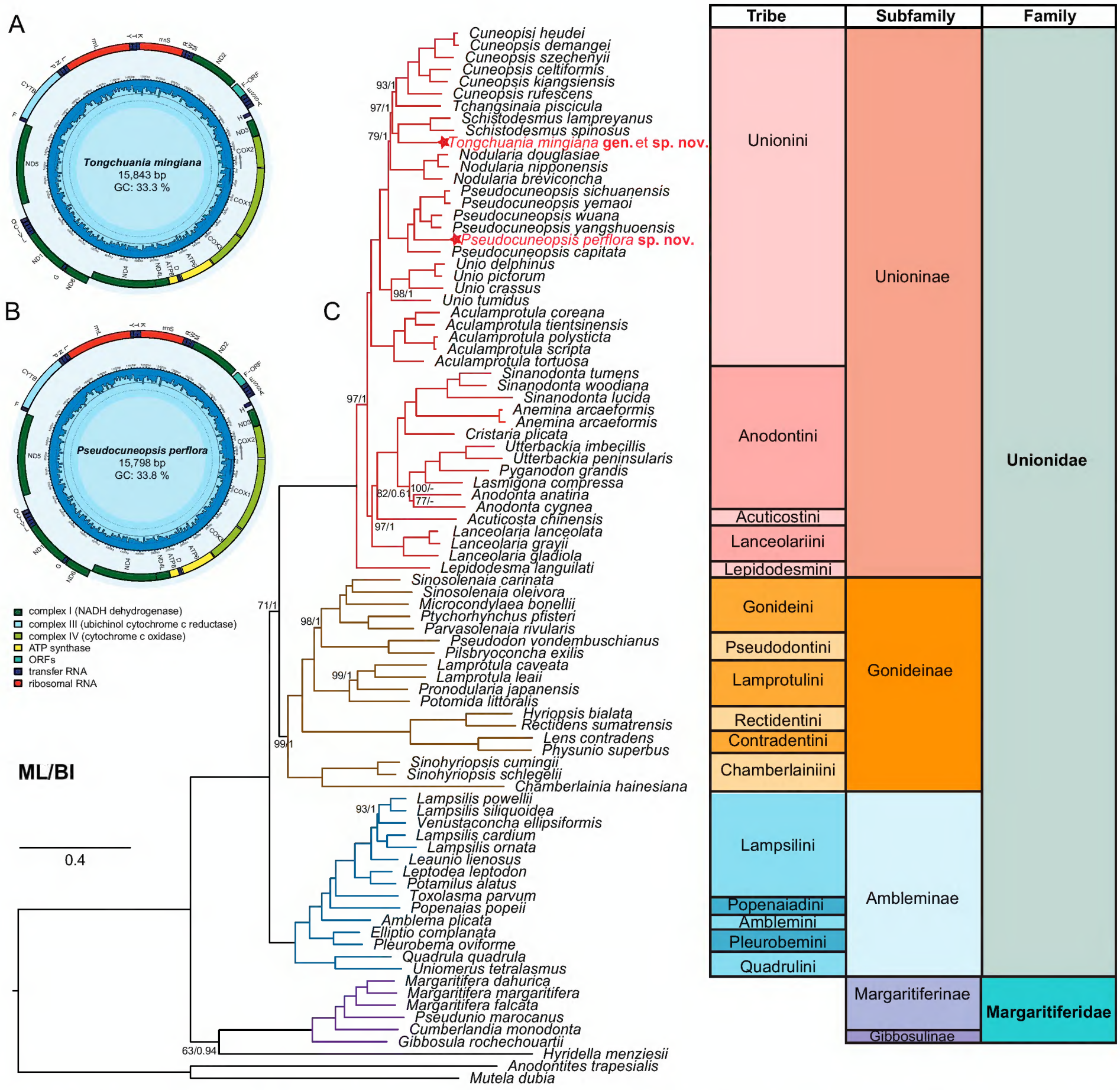

Figure 3. Analysis based on mitochondrial data. **A, B.** Gene map of the F-type mitochondrial genome of *Tongchuania mingiana* gen. et sp. nov. and *Pseudocuneopsis perflora* sp. nov.; **C.** Maximum likelihood and Bayesian inference tree inferred from mitochondrial genome sequences. Nodes with bootstrap (ML) = 100 and posterior probability (BI) = 1 were omitted; conflicts between BI and ML trees were marked with '-'.

sister taxon (PP = 1.0, BS = 76), with *P. capitata* positioned as the sister lineage to the remaining clade (PP = 1.0, BS = 76). The overall relationship can be represented as: (((*P. sichuanensis* + *P. yemaoi*) + (*P. wuana* + *P. yangshuoensis*)) + *P. perflora* sp. nov.) + *P. capitata*.

Systematics

Family Unionidae Rafinesque, 1820
Subfamily Unioninae Rafinesque, 1820
Tribe Unionini Rafinesque, 1820

Genus *Tongchuania* H. Chen, H.-Q. Xiang, Y.-M. He, X.-C Huang & X.-P. Wu, gen. nov.

https://zoobank.org/B4B02530-17E1-435A-BCDB-3DA8B898A161

Type species. *Tongchuania mingiana* H. Chen, H.-Q. Xiang, Y.-M. He, X.-C Huang & X.-P. Wu, gen. et sp. nov. (Fig. 4).

Diagnosis. Shell medium-sized, thick, solid, glossy, yellowish-brown, overall slender shape; anterior margin slightly indented, ventral margin nearly straight, dorsal margin straightened; periostracum with straight blackish-green rays; umbo sculptured with W-shape ridges. Both left and right valves with two pseudocardinal teeth. Nacre white.

Description. Shell medium-sized, thick, solid, glossy, yellowish-brown, overall slender shape; anterior margin regularly rounded and slightly indented, ventral margin nearly straight, dorsal margin straightened; periostracum with straight blackish-green rays; umbo located at 1/3 of shell length and obviously higher than the dorsal margin; umbo sculptured with W-shape ridges. Ligament long, narrow. Anterior adductor muscle attachment elliptical; posterior adductor muscle attachment round and smooth. Both left and right valves with two pseudocardinal teeth. Left valve with two pseudocardinal teeth, anterior tooth wide, posterior tooth slightly thick and acute triangle, and having two lateral teeth. Right valve with two thick elevated pyramidal pseudocardinal teeth and one lateral tooth. Nacre white, and shiny (Fig. 4). Within the incurrent aperture, there are not obvious papillae arranged in one row, with brown pigmentation; papillae of the excurrent aperture well developed, stubby, and arranged in one row, with brown pigmentation. The inner gills are larger than the outer gills. Labial palps are thin and small, without pigmentation (Fig. 5A, B).

Etymology. The name of the genus originates from the ancient Chinese name for Dazhou.

Vernacular name. 通川蚌 (tong chuan bang) (Chinese); Tongchuan mussel (English).

Remarks. The species of this genus is similar only to those in the genus *Nodularia* within the Tribe Unionini, but it can be easily distinguished by its periostracum with straight blackish-green rays and the right valve with two thick, elevated pyramidal pseudocardinal teeth.

Tongchuania mingiana H. Chen, H.-Q. Xiang, Y.-M. He, X.-C Huang & X.-P. Wu, gen. et sp. nov.

https://zoobank.org/993281B6-06BA-4676-B84C-2A537C28EF5A

Material examined. ***Holotype*:** NCUTM250511, shell length 54.27 mm, shell width 19.23 mm, shell height 28.97 mm (Fig. 4A), Bahe River, Dazhou City, Sichuan Province, China, 31.0978°N, 107.1856°E, in April 2025.

***Paratypes*:** 5 specimens, NCUTM250512–16, shell length 51.02–79.08 mm, shell width 18.34–27.33 mm, shell height 29.03–41.22 mm (Fig. 4B, C), locality and habitat same as holotype.

Diagnosis. Same as the genus.

Description. Same as the genus.

Etymology. The specific epithet "*mingiana*" refers to the highly distinctive yellow-green band on the shell (a diagnostic character clearly distinguishing this species from other genus in the tribe Unionini), derived from the Chinese "mingiana" (bright mirror), alluding to the vivid band's mirror-like contrast against the darker shell background.

Vernacular name. 明鉴通川蚌 (ming jian tong chuan bang) (Chinese); Brightband Tongchuan mussel (English).

Habitat and distribution. The species is currently only found in the slower-flowing sections of the Bahe River in Dazhou City, Sichuan Province. It lives in crevices between stones on gravel riverbeds at a depth of 0.5 to 1 meter (Fig. 1B).

Genus *Pseudocuneopsis* Huang, Dai & Wu, 2022

Type species. *Pseudocuneopsis capitata* (Heude, 1874).

Pseudocuneopsis perflora H. Chen, H.-Q. Xiang, Y.-M. He, X.-C Huang & X.-P. Wu, sp. nov.

https://zoobank.org/48D7468A-AE26-4504-B4F0-A5F0703115CC

Material examined. ***Holotype*:** NCUPP250521, shell length 61.02 mm, shell width 20.11 mm, shell height 36.11 mm (Fig. 6A), Bahe River, Dazhou City, Sichuan Province, China, 31.0978°N, 107.1856°E, in April 2025.

***Paratypes*:** 6 specimens, NCUPP250522–27, shell length 44.17–63.19 mm, shell width 14.86–19.91 mm, shell height 25.00–35.09 mm (Fig. 6B, C), locality and habitat same as holotype.

Diagnosis. Shell medium-sized, flat, thick, solid, yellowish-brown without any color rays; anterior margin rounded, ventral margin nearly straight; dorsal margin slightly curved and truncated behind, with an obtuse angle in the middle of posterior margin; shell surface with a low secondary posterior ridge end in the angle on the posterior margin; umbo sculptured with W-shape ridges. Both left and right valves with one or two pseudocardinal teeth. Nacre white.

Description. Shell medium-sized, flat, thick, solid, yellowish-brown without any color rays; anterior margin rounded, ventral margin nearly straight; dorsal margin slightly curved and truncated behind, with an obtuse angle

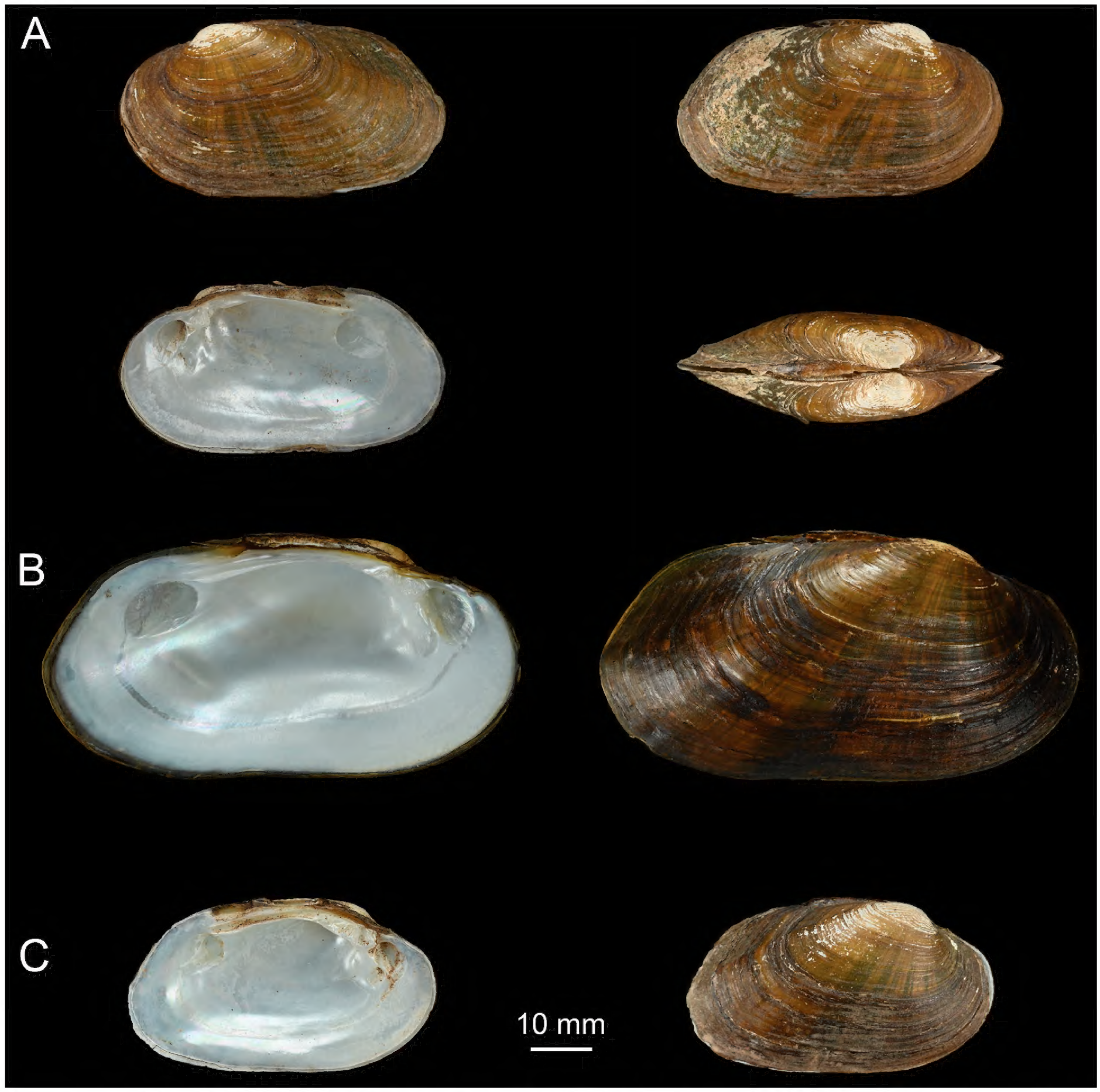

Figure 4. *Tongchuania mingiana* gen. et sp. nov. **A.** Holotype, NCUTM250511; **B, C.** Paratypes, NCUTM250512, 13. Scale bars: 10 mm.

in the middle of posterior margin; shell surface with a low secondary posterior ridge end in the angle on the posterior margin; umbo sculptured with W-shape ridges. ligament long, narrow. Anterior adductor muscle attachment elliptical and deep; posterior adductor muscle attachment round. Left valve with two pseudocardinal teeth, anterior tooth wide, posterior tooth slightly thick, and having two lateral teeth. Right valve with one thick elevated pyramidal pseudocardinal tooth and one lateral tooth. Nacre white, and shiny (Fig. 6). Within the incurrent aperture, there are not obvious papillae arranged in one row, without pigmentation; the excurrent aperture without papillae, smooth, with brown pigmentation. The inner gills are larger than the outer gills. Labial palps are thin and small, with brown pigmentation.

Etymology. "per-" means "Lasting, long-lasting", and the root word flora means "luxuriant". The type locality (Bahe River, Dazhou City) maintains pristine aquatic conditions, and the name conveys a wish for the species' persistent prosperity.

Vernacular name. 永盛伪楔蚌 (yong sheng wei xie bang) (Chinese); Everlasting pseudo-wedge mussel (English).

Habitat and distribution. The species lives alongside *Tongchuania mingiana* gen. et sp. nov. in its river section, where other freshwater mussel species are rarely found (Fig. 1B).

Remarks. The dorsal margin is slightly curved and truncated at the rear, with an obtuse angle in the middle of the posterior margin. The shell surface features a low secondary posterior ridge that ends at the angle on the posterior margin, which helps distinguish this species from others in the same genus. Additionally, the umbo is sculptured with dense W-shaped ridges, a feature not found in other species of the genus.

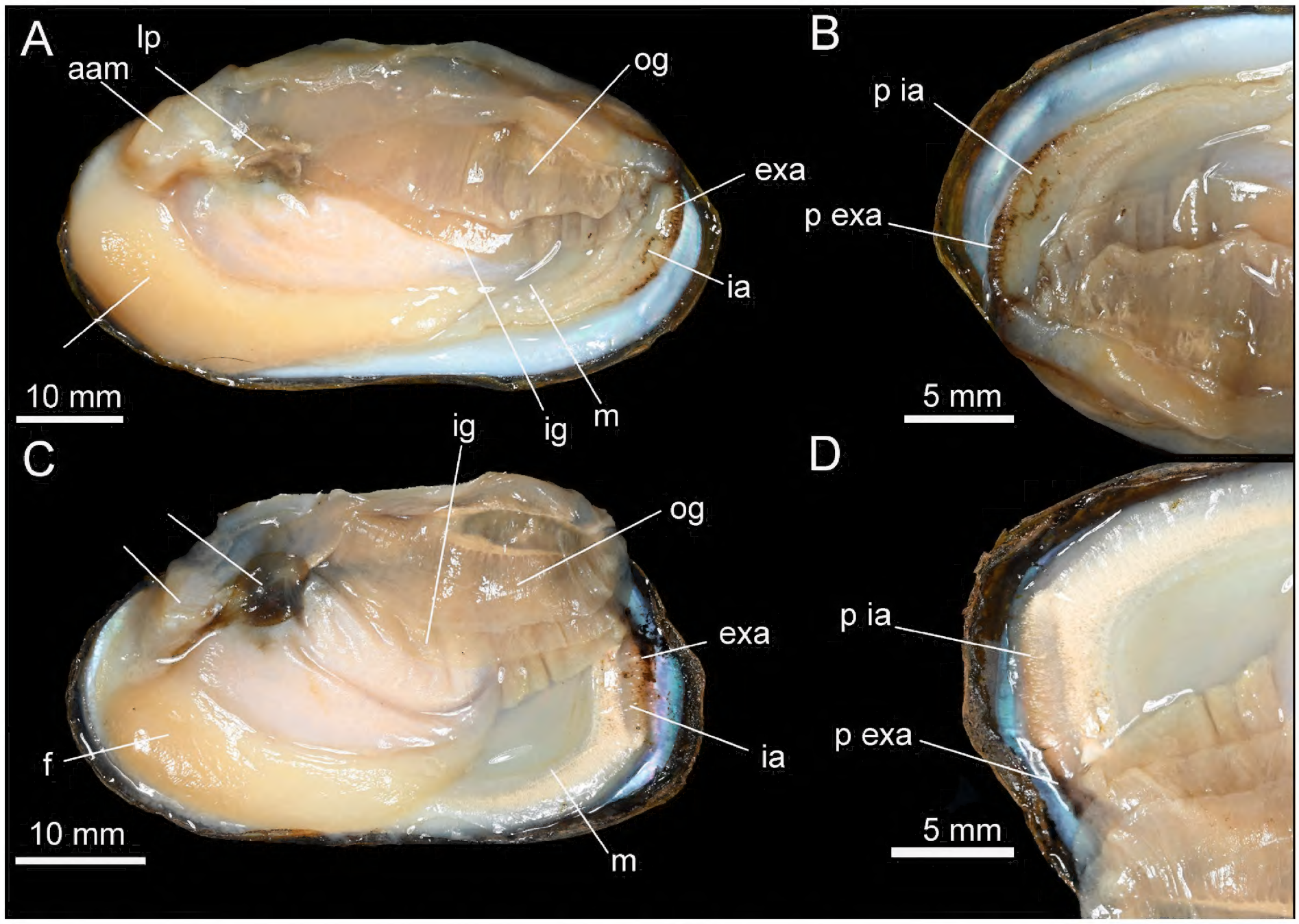

Figure 5. A, B. Anatomical features of *Tongchuania mingiana* gen. et sp. nov. **C, D.** Anatomical features of *Pseudocuneopsis perflora* sp. nov. Abbreviations: aam, anterior adductor muscle; exa, excurrent aperture; ia, incurrent aperture; f, foot; ig, inner gill; og, outer gill; lp, labial palps; m, mantle; p ia, papillae in incurrent aperture; p exa, papillae in excurrent aperture.

Discussion

The genus *Pseudocuneopsis* likely harbors significantly greater species diversity than currently recognized, although our understanding of its phylogeny remains constrained by insufficient sampling across key river basins in southwestern China (Wu et al. 2022; Liu et al. 2023; Dai et al. 2024a). To date, only five valid species have been formally described (Dai et al. 2024a), yet phylogenetic evidence strongly indicates the existence of multiple undescribed lineages awaiting formal recognition. Notably, our analyses reveal a discordant biogeographic pattern within the Sichuan Basin, where phylogenetic relationships do not mirror geographic proximity. Although *P. sichuanensis* (from Baitiao River, Chengdu, Sichuan) and *P. perflora* sp. nov. (from Bahe River, Dazhou, Sichuan) are found in spatially adjacent tributaries within the Sichuan Basin, they do not represent sister lineages. Instead, *P. sichuanensis* shows closer affinity to *P. yemaoi* from the distant Han River basin (Xiangyang, Hunbei), whereas *P. perflora* sp. nov. occupies a position relative to other congeners. This phylogenetic placement suggests an early divergence event, possibly reflecting cryptic diversification within the upper Yangtze or an ancestral colonization event predating the radiation of the Guangxi and Hubei/Sichuan groups. The potential unrecognized diversity within *Pseudocuneopsis* underscores the urgent need for comprehensive field surveys across unsampled tributaries, coupled with integrative taxonomic approaches, to uncover cryptic lineages and refine our understanding of the evolutionary history of freshwater mussels.

The Sichuan Basin may be a potential hotspot for diversity of bivalve species. We establish one new freshwater mussel genus and two new species (Unionidae, Unioninae, Unionini) through integrative taxonomic analysis combining detailed morphological, anatomical, and molecular systematic approaches. A total of six endemic species have been formally recorded from Sichuan Basin. In addition, in recent years, a new genus and two new species were also found in the same river (Baotiao River) in the western Sichuan Basin (Wu et al. 2022, 2025). There are many rivers in the Sichuan Basin and the investigation was inadequate and we speculate that there are still many survey gaps and more new species to be discovered.

Our findings highlight the urgent need for comprehensive surveys in understudied Asian freshwater ecosystems, where species may face extinction before being documented. Due to the significant decline in the number of Unionidae species worldwide, understanding their phylogenetic issues is crucial for the conservation of these vulnerable species (Mace 2004; Lopes-Lima et al. 2017; Saito et al. 2022). For the two new species described in this paper,

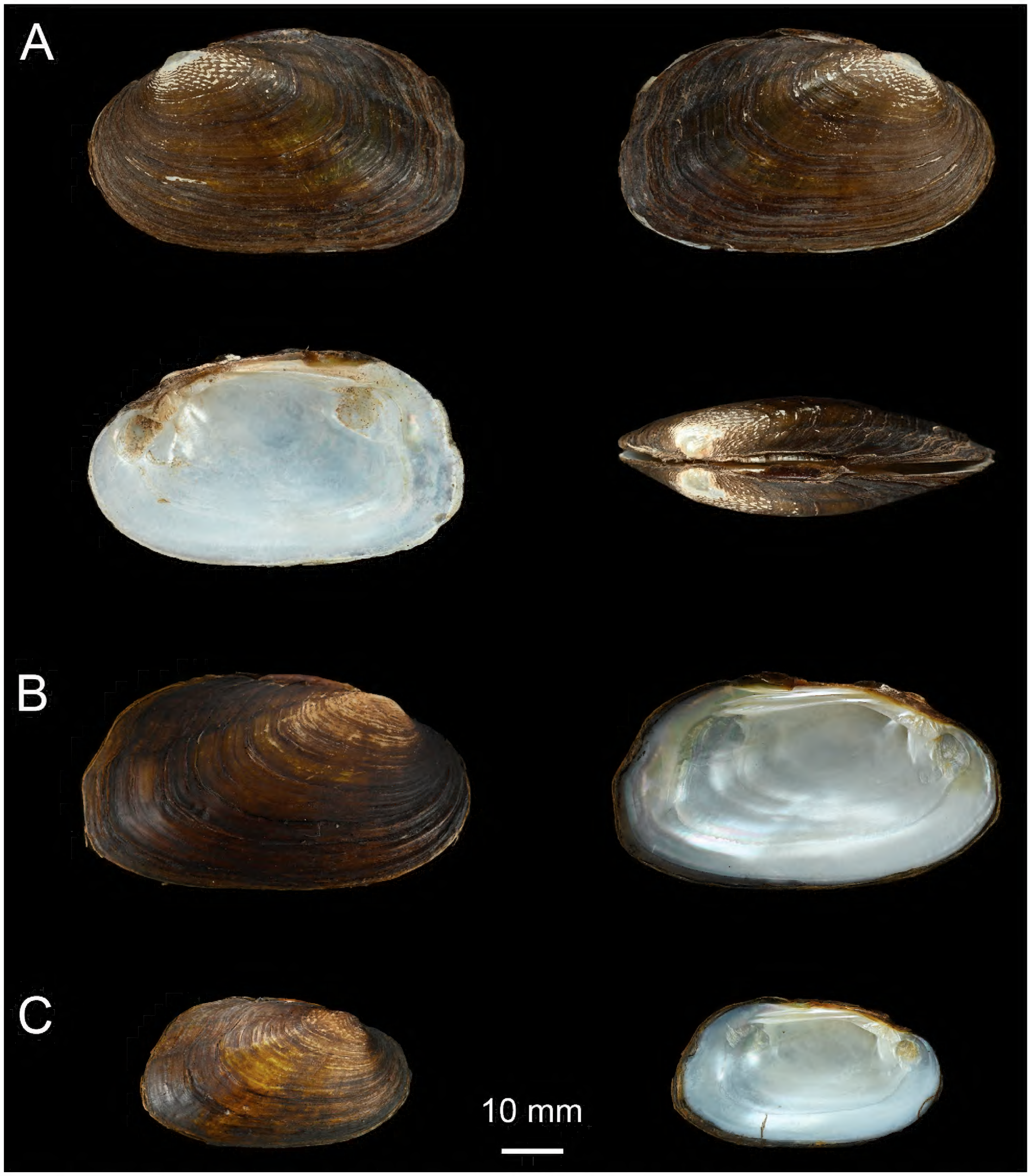

Figure 6. *Pseudocuneopsis perflora* sp. nov. **A.** Holotype, NCUPP250521; **B, C.** Paratypes, NCUPP250522–27. Scale bars: 10 mm.

their survival status is concerning, as no living individuals have been found outside the Bahe River, and only empty shells were discovered in other rivers around Dazhou. This study suggests that these species were likely widely distributed across surrounding river systems in the past, but their habitats have been drastically reduced in recent times due to riverway destruction (during the survey, it was found that many rivers in Sichuan Province have been altered due to urban construction). Moreover, two other endemic species in the Sichuan Basin—*Acuticosta sichuanica* (Zeng & Liu, 1989) and *Parvasolenaia triangularis* (Heude, 1885)—have not been observed alive since their initial descriptions. Their apparent disappearance may be linked to river habitat degradation and the escalating effects of extreme climatic events (Bolotov et al. 2018; Liu et al. 2020; Lopes-Lima et al. 2020). These findings emphasize the urgent need to protect micro-endemic habitats within geologically complex basins where specialized species face heightened extinction risks from anthropogenic and environmental pressures.

The application of molecular techniques has significantly advanced our understanding of phylogenetic

relationships among freshwater bivalves (Huang et al. 2025). This progress is particularly crucial for taxa inhabiting similar environments, where convergent morphological adaptations often obscure true evolutionary relationships. A striking example is the morphological comparison between *Pseudopostolata* and *Postolata*, although these genera are taxonomically distinct at the generic level, they exhibit remarkable shell similarities (Dai et al. 2024a; Liu et al. 2024). Such morphological convergence frequently leads to taxonomic misclassification when relying solely on shell characteristics, as demonstrated by Wu et al (2025). Despite belonging to different subfamilies, the shells of *P. perflora* sp. nov. and *Pseudopostolata* exhibit similarity in shell shape. This finding reinforces the well-documented limitations of shell-based identification in freshwater mussels (He and Zhuang 2013; Bogatov and Prozorova 2017). By integrating molecular data with traditional morphological approaches, this study is uncovering previously unknown diversity. These methodological advances are critical for global conservation efforts, particularly for ecologically important but highly vulnerable freshwater bivalves. Accurate species delimitation through molecular techniques provides an essential foundation for effective protection strategies, especially for taxa facing imminent extinction threats in rapidly degrading freshwater ecosystems.

Data availability statement

The data that support this study are available in the article and accompanying online Supplementary material.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Declaration of funding

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Supplementary material 1

Supplementary information

Authors: Hui Chen, Hong-Quan Xiang, Yu-Ting Dai, Zhong-Guang Chen, Yue-Ming He, Ge Guo, Xiao-Ping Wu, Xiao-Chen Huang

Data type: docx

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